The Patent Office

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

GB99/3721

4

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

[redacted]e-registration under the Companies Act does not constitute a new legal entity but merely [redacted]bjects the company to certain additional company law rules.

Signed R. Mahoney

Dated 17 November 1999

Patents Form 1/77

Patents Act 1977
(R 6)

The Patent Office

Request for the grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

12 JAN 1999

1. Your reference

REP05997GB

2. Patent application number
(The Patent Office will fill in this part)

9900708.0

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Microscience Ltd.
67-68 Jermyn Street
London
SW1Y 6NY
United Kingdom

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

75836850001

4. Title of the invention

VIRULENCE GENE AND PROTEIN, AND THEIR USE

5. Name of your agent *(if you have one)*

GILL JENNINGS & EVERY

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Broadgate House
7 Eldon Street
London
EC2M 7LH

Patents ADP number *(if you know it)*

745002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	4
Claim(s)	1
Abstract	
Drawing(s)	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. For the Applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature

Date 13 January 199

12. Name and daytime telephone number of person to contact in the United Kingdom

PERRY, Robert Edward
0171 377 1377

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

VIRULENCE GENE AND PROTEIN, AND THEIR USE

Field of the Invention

This invention relates to a virulence gene and protein, and their use. More particularly, it relates to their use in therapy and in screening for drugs.

Background of the Invention

E. coli is an organism that is implicated in septicaemia, meningitis, urinary tract infection, wound infection, abscess formation, peritonitis and cholangitis. It would be desirable to provide means for treating or preventing conditions caused by *E. coli*, e.g. by immunisation.

In prokaryotes glycosyltransferases transfer activated sugars to a variety of substrates including glycogen, fructose-6-phosphate, lipopolysaccharide and capsular polysaccharide. Members of the group 1 glycosyltransferases transfer either UDP, ADP, GDP or CMP linked sugars.

Summary of the Invention

The present invention is based on the discovery of a virulence gene in *E. coli* K1, which has been designated *eck1*, that encodes a protein with homology to several group 1 glycosyltransferases from a number of bacteria. Accordingly, the present invention provides:

The therapeutic use of a peptide encoded by the operon including the *eck1* gene in *E. coli* K1, or a homologue thereof in a Gram-negative bacterium, or a functional fragment thereof, e.g. a peptide comprising all or part of the 150-member amino acid sequence defined below;

a host transformed to express the peptide or modified to disrupt expression of the gene;

a vaccine comprising such a peptide or the means for its expression, or an attenuated vaccine in which the virulence gene is disrupted;

the use of the peptide or corresponding polynucleotide as a target for screening potentially useful drugs, especially anti-microbials, or as a diagnostic agent in the detection of virulence, e.g. for testing for the presence of virulent coliforms in livestock.

Description of the Invention

The virulence gene in *E. coli* K1 was identified by using signature-tagged mutagenesis (STM) to screen an *E. coli* K1 mini-Tn*5* mutant bank for attenuated mutants, in a mouse model of systemic infection. Bacteria containing a mini-Tn*5* insertion within the virulence gene failed to be recovered from mice inoculated with a mixed population of mutants, and are therefore likely to be attenuated.

The cloned *E. coli* K1 nucleotide sequence following the mini-Tn*5* insertion is as follows:

Length: 454

```
   1  TGATTTTTGA GATAATAACT GACAATCTCT AATTTCAAAC AAACAACCAT
  51  TATAGCCATC TTCTATTAAG CTATTATTAC CTGGAATATT AGTGACTATA
 101  CATGGAAGTC CACAGCTCAA TGCTTCTAAA ATTGCTAATG GCATACCCTC
 151  CCAAAGAGAA GGTAATATAA AAAGATCATT AACTTTTAAA ATATTAACAA
 201  TGTTATCTGA CCATCCATGA AAAATTATAC GTCCATCTTG CCGTTTGAAC
 251  CTGCTTTCTA ACTGTTCTTT TAGTTCACCA TCTCCTACAA GTGTCAGCTT
 301  AACATTAACA TTTTCATTCA GCAGTTTTTC AACAGCAAGC AATAATGTCT
 351  CAGGATCTTT TTGCTTGGAT AATCTACCAA CCATTACTAG ATTCAAGGTG
 401  CTACTATAAA TTTTATTTTC TAAAGGAGAA AACTTATCAG TGTCTACTCC
 451  ATTA
```

A translation of this sequence is as follows:

Length: 150 amino acids

```
   1  GVDTDKFSPL ENKIYSSTLN LVMVGRLSKQ KDPETLLLAV EKLLNENVNV
  51  KLTLVGDGEL KEQLESRFKR QDGRIIFHGW SDNIVNILKV NDLFILPSLW
 101  EGMPLAILEA LSCGLPCIVT NIPGNNSLIE DGYNGCLFEI RDCQLLSQKS
```

This amino acid sequence shows 37.5% identity to the *epsG* protein of *Streptococcus thermophilus*, (accession number Q56044) at amino acids 197-326 of the latter.

GCG bestfit analysis at the amino acid level is as follows.

```
197 MVGRLSPPKEFFFFIDFAKKILQIRNDTNFIIVGDGELRSEIERMILDNG 246
    ||||||  |:    :   .|:|    .    :|||||: ::|
 23 MVGRLSKQKDPETLLLAVEKLLNENVNVKLTLVGDGELKEQLESRF..KR 70

247 LGDKIYITGWVDNPRNYIEKFDQAILFSRWEGLSLTIAEYMSQKKTILAT 296
      :|   || ||  | :.  |  || | |||: | | | :|     : |
 71 QDGRIIFHGWSDNIVNILKVNDLFILPSLWEGMPLAILEALSCGLPCIVT 120

297 NIGGINDLITDGETGMLIEVGDLNSAVSKS 326
    || | | || ||  | | |: |      ||
121 NIPGNNSLIEDGYNGCLFEIRDCQLLSQKS 150
```

The 150 amino acid sequence also shows 33% identity to a hypothetical protein from *Synechosystis* spp. (accession number P73948)

GCG bestfit analysis at the amino acid level is as follows

```
177 GVAVERYCPGQNDLKKEYQAERLFIYLGRIAPEKNVEALLKG.WKFSDMG 225
    ||  ::: | :|   | | .    : .||:. :|. | ||    |  .
  1 GVDTDKFSPLEN...KIYSSTLNLVMVGRLSKQKDPETLLLAVEKLLNEN 47

226 PHCKLLMVGDGILKSTLQTHYGPEMGVHWLGFVADELTRIQLLRAADAFI 275
     . || :|||| ||  |:. :  : |       .| :  : :|:  | ||
 48 VNVKLTLVGDGELKEQLESRFKRQDGRIIFHGWSDNI..VNILKVNDLFI 95

276 LPSLVEGLSLSLLEAMACGTACVATDAGADGEVLENG 312
    |||| ||: |.:|||:.||  |: |.   .  .:|.|
 96 LPSLWEGMPLAILEALSCGLPCIVTNIPGNNSLIEDG 132
```

The 150 amino acid sequence also shows homology to proteins from a wide range of prokaryotes. These proteins are listed in the glycos_transf_1 family in the PFAM database of multiple protein alignments (accession number PF00534). These proteins include the hypothetical *Klebsiella pneumoniae* protein YC07 (SwissProt accession number Q48453), the putative colanic acid biosynthesis glycotransferase WcaL from *E.coli* K12 (SwissProt accession number P71243) and the hypothetical *bacillus subtilis*

protein YPJH (SwissProt accession number P42982).

The novel gene has been tested for attenuation of virulence, using mixed infections, in a murine model of systemic infection (Achtman *et al.*, 1983, Infection and Immunity, vol 39, pages 315-335), and shown to be attenuated with a competitive index (CI) of 0.025 (mean CI from three mice).

The *E.coli* K1 *eck1* gene is likely to be useful both in generating attenuated vaccine strains and as a target for antimicrobials.

For the purposes of this invention, the appropriate degree of homology is typically at least 50%, preferably at least 60% or 70%, and more preferably at least 80% or 90% (at the amino acid or nucleotide level).

It is evident that *E. coli* K1 strains containing disruptions of the invention are attenuated. The products of the invention may be immunogenic. They are therefore useful in therapy, and more particularly as a prophylactic, in a vaccine.

The protein may be purified. It may be sequenced. The corresponding full-length gene can thus be identified. It can thus be prepared by recombinant technology, by expression in a suitable host. Active fragments and homologues can be identified. Vaccine compositions, including attenuated vaccines, can be formulated, with carriers and adjuvants as necessary or desired, and used in therapy, to provide an effective immunisation against *E. coli*. In some cases, antibody may be used, for passive immunisation. All these procedures are known to those of ordinary skill in the art, and do not affect the nature of the invention that has been made.

CLAIMS

1. A peptide encoded by the operon including the *ecK1* gene *E. coli* K1, or a homologue thereof in a gram-negative bacterium, or a functional fragment thereof, for therapeutic use.

2. A peptide according to claim 1, comprising the 150-member amino acid sequence defined herein.

3. A polynucleotide encoding a peptide according to claim 1 or claim 2, for therapeutic use.

4. A host transformed to express a peptide according to claim 1 or claim 2.

5. A vaccine comprising a peptide according to claim 1 or claim 2, or the means for its expression.

6. A vaccine comprising a microorganism having a virulence gene deletion, wherein the gene encodes a peptide according to claim 1 or claim 2.

7. Use of a product according to any of claims 1 to 4, for screening potential drugs or for the detection of virulence.

8. Use of a product according to any of claims 1 to 4, for the manufacture of a medicament for use in the treatment or prevention of a condition associated with infection by *E. coli*.

PCT NO : GB99/03721

Form 23/77 : 9.11.99

AGENT : Gill Jennings & Every